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Assessment of molecular diversity and tolerance of increased salinity levels in irrigation of barley seedlings

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ABSTRACT

Salinity is one of the biggest challenges that limit the productivity of crops. Therefore, Screening for more tolerant varieties is a top priority of breeders. Landraces are great source for genetic variability that would be used for improvement of new varieties. Thus, in this study five barley landraces genotypes were collected from different locations in the New Valley governorate of Egypt and two check varieties, Giza 123 (salt tolerant) and Beecher (salt sensitive) as a control were evaluated for their potentiality to salt stress tolerance and genetic variability, via exposure of seedlings to series graded salt concentrations course and inter simple sequence repeat (ISSR) molecular marker analysis. Survived seedlings were counted under both non-stress and stress conditions. Highly significant differences were observed among genotypes under salt stress conditions, whereas all genotypes were affected by salinity stress. Seedlings mortality varied among genotypes, where salt sensitive check (Beecher) variety was highly affected. While, L2 had the lowest seedlings death rate and distinct over salt tolerant check variety (Giza 123). Furthermore, ISSR molecular marker techniques were used for study of genetic diversity and similarity relationships among the seven genotypes. The polymorphism was 86.89%. L1 and L5 were the highest similar (0.74) genotypes, but the lowest similarity (0.45) was noted between L4 and Giza 123 genotypes. Moreover, dendrogram analysis gathered the highest salt tolerant genotypes in one group. Superiority of L2 genotype in comparison with check salt tolerant variety suggests that landraces could be beneficial tool for genetic improvement of barley stress tolerant lines.

Keywords: landraces, Seedling, Barley (*Hordeum vulgare* L.), salinity tolerance, molecular markers.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cultivated cereal grain in the world. It is belongs to grass family and have a diploid state (2n = 2x = 14), with a large chromosomes (Hori *et al.*, 2005). Furthermore, It is considered as substantial source for animal food and human feeding in many populations around the world (Newman and Newman, 2006). In Egypt, barley is cultivated in an area of 208849 feddans with an annual production of 117113 ton in 2010 (AERMAE 2011). This productivity is limited by many factors such as salinity which considers as one of the vastest environmental stresses whereas, more than 33% out of total cultivated area in Egypt is suffering from salinity (Khatab and Samah, 2013). Moreover, salinity has adverse effects on plant different growth stages such as germination, plant growth, crop productivity and causing plant death at high levels (Parida and Das, 2005; Dadashi, 2008). Through impairing many vital plant processes such photosynthesis, transpiration and other biochemical pathways (Kendirli *et al*., 2005 and Tiwari *et al*., 2010). However, there is an essential need to improve barley productivity to overcome the huge demand and consumption. The expansion of cultivated area in the new reclaimed lands needs to grow the most tolerant genotypes for a biotic stress. Therefore, genetic improvement approach is necessary to generate new more tolerant genotypes. In this respect, landraces are one of useful sources for genetic variability that would be used in scientific breeding programs for improving the productivity of new crop varieties (Ceccarelli, 1994 and Newton *et al*., 2010). Screening for salt tolerant genotypes is depending on several parameters which widely done during germination and seedling stages (Yousofinia *et al*., 2012; Sbei *et al*., 2014 and El-Hamamsy and Behairy, 2015).

Nowadays, molecular markers especially DNA genetic markers have been used in large scale for assessing of genetic diversity among genotypes. Inter simple sequence repeats or ISSR one of the widespread used PCR based molecular markers (Fernández *et al.*, 2002).

In this study, five barley landraces and two check varieties were screened for their ability to salinity stress tolerance and genetic variability via exposure of seedlings to salinity course and ISSR molecular marker analysis.

MATERIALS AND METHODS

Plant material:

Five barley (*Hordeum vulgare* L.) landraces namely, L1, L2, L3, L4 and L5 were collected from farmers from different locations in the New Valley governorate of Egypt (Table 1), and the two check varieties i.e., Giza 123 (salt tolerant) and Beecher (salt sensitive) were used in this study. These two genotypes were provided by Agricultural Research Center, Egyptian ministry of Agriculture**.** Seeds of each genotype were sown in 6 plastic pots with a diameter 30 cm containing a mixture of Soil and vermiculite (3:1 v/v) and irrigated with tap water under green house conditions. After four days of germination, seedlings density was thinned to 15 seedlings per pot. Then, the total of 42 pots was divided into two groups each of them contained 21 pots, three pots for each genotype and they were distributed randomly. One group was irrigated with tap water (as a control), while the other group watered with series salt concentrations course for each pot which started with 50 mM NaCl for two days, then the NaCl concentration was increased gradually (100, 150, 200, 250 or 275 mM) for three days for each of the three initial concentrations, then followed by six

days for 250 mM concentration and three days for the last one (275 mM). After 20 days of salinity treatment, the dead seedlings rate was recorded. The stress susceptibility index (SSI) was calculated using following formula (Fischer and Maurer, 1978): SSI= [1-(xs/xp)]/[1-(Xs/Xp)], Where, xp and xs are the seedlings survival rate of the genotype under non-stress and stress conditions, respectively. While, Xs is mean values of the seedlings survival rate of all the genotypes under stress conditions but Xp under non-stress condition.

The collected data were statistically analyzed using randomized complete design (RCD) with a three replications. Mean comparisons were performed using Least Significant Differences (L.S.D) test at 5% and 1% levels of probability according to SAS-program (Der and Everittt, 2009).

Table 1. The names and original place of collection of the studied five barley landraces.

No	Original place of collection			
	Name	Location	Province	Region
1	L1	Balat	The New Valley	Southwest Egypt
2	L2	Mout	The New Valley	Southwest Egypt
3	L3	Elrashda	The New Valley	Southwest Egypt
4	L4	Alqasr	The New Valley	Southwest Egypt
5	L5	Elshewash	The New Valley	Southwest Egypt

Molecular characterization

DNA extraction: small fresh leaves were used for extraction of genomic DNA by using a modified CTAB method as revealed by Ben El Maati *et al*., (2004).

PCR amplification and electrophoresis: nine primers of ISSR markers (UBC 826, UBC 812, UBC 846, UBC 807, UBC 808, UBC 810, UBC 811, UBC 816 and UBC 861) from EZBiolab-USA were utilized in this study. PCR amplification reaction was carried out as shown by Ben El Maati *et al*., (2004). 1.5% agarose gels were used for separation of PCR products, followed by ethidium bromide staining to visualize amplified DNA fragments. The detected bands were recorded as 1 (present) and 0 (absent). Nei-Li's similarity index was used for estimation of genetic similarity (Nei and Li, 1979). A dendrogram was carried out according to the similarity matrix data by unweighted pair group method with arithmatic average (UPGMA) using MEGA program software. This program was also used in accomplishment of cluster analysis.

RESULTS AND DISCUSSIONS

A. Salinity tolerance evaluation:

To study the tolerance ability of collected landraces and the two check varieties for salinity stress, it were subjected to series levels of NaCl at seedling stage and the survived seedling rate was counted. The analysis of variance showed that highly significant variations among genotypes in the survived seedling rate (Table 2). According the mean performance of the all genotypes, under non-stress condition the all seedlings of all genotypes were alive and the mean rate of all genotypes were 100 %, While under severe salinity stress the rate of survived seedlings was varied among genotypes and ranged from 24.9 (Beecher) to 80 % (L2). Moreover, L2 genotype was more tolerant than salt tolerant check variety (Giza 123) (Table 3). In this study screening for salinity was done at seedling stage as performed by many researchers (Dadashi, 2008; Yousofinia *et al*., 2012; Tabatabaei, 2013; Sbei *et al*., 2014). Also, it was observed that the high level of salt concentration increased seedlings death rate as similar with results of Dadashi (2008) who observed that low levels of salinity reduced seedlings shoot growth while at an excessive level significantly reduced seedlings survival rate. However, one of possible reasons for seedlings death under high salt concentration which gives oxidative stress may be due to more accumulation of reactive oxygen species (ROS), which cause cell damage (Vaidyanathan *et al*., 2003; Foyer and Noctor, 2005). Therefore, salinity is impairing many vital plant processes such photosynthesis, transpiration and other biochemical pathways (Kendirli *et al*., 2005 and Tiwari *et al*., 2010).

Salinity tolerance estimation was shown in Table (3), the all genotypes categorized into three groups, high salinity tolerance (HST) group which contained two genotype (Giza 123 and L2) had lower rate for salinity sensitivity index (0.49 and 0.42) and survived seedlings rate (77.5 and 80.0 %), respectively. Moderate salinity tolerance (MST) group included genotypes displayed moderate values in the seedlings mortality. The two genotypes (Beecher and L4) which had higher values for salinity sensitivity index (1.6 and 1.5) and survived seedlings rate (24.9 and 27.6 %), respectively were grouped in the last low salinity tolerance (LST) group.

However, using of stress susceptibility index through comparing normal and stress conditions was more useful parameter for detection of more tolerant genotypes (Khodarahmpouret *et al.*, 2011 and Sbei *et al*., 2014).

Table 2. Mean squares for survived seedlings rate of five barley landraces and two check varieties in response to salinity stress.

S.O.V	D.F	Survived seedlings
Genotype	6	1397.76**
Error	14	1.31

***and ** indicate significant at 5% and 1% levels of probability, respectively**

Table 3. Percentage of survived seedlings and salinity sensitivity index (SSI) in five barley landraces and two check varieties under normal and salinity conditions.

genotypes	% survived seedlings		Salinity sensitivity index (SSI)	Tolerance degree
	Normal condition	Salinity condition		
Giza 123	100	77. 50	0.49	High
L1	100	55.17	0.94	Moderate
L2	100	80.00	0.42	High
L3	100	58.20	0.89	Moderate
L4	100	27.57	1.50	Low
L5	100	55.70	0.94	Moderate
Beecher	100	24.90	1.60	Low

L, refers to name of barley landraces

B- ISSR marker analysis:

To discriminate genetically among evaluated genotypes, nine ISSR primers were utilized in detection of several DNA bands from genomic DNA (Fig. 1). The size of all detected bands was in between 131 bp and 1240 bp with an average 8.7 bands per primer (Table 4). The maximum number of bands (13 bands) was given by UBC 861 primer and the highest percentage of polymorphism was obtain by UBC 846, UBC 816 and UBC 861 (100 %) for each. While UBC811 primer obtained the minimum bands (6 bands). The total of all obtained bands was 78 bands, 69 of them were polymorphic, with an average of 7.7 polymorphic bands per genotype. The polymorphism proportion extended from 71.43% to 100% with an average of 86.89% (Table 4). Genetic diversity among barley genotypes via ISSR marker analysis was also performed by El-Awady *et al*. (2012); Khatab and Samah (2013); Akladious and Abbas (2014). Obviously, high polymorphism rate was detected among genotypes and it is consist with several studies, Hou *et al*. (2005) obtained 98.1 % polymorphism rate, while 92.2 % was observed by Eshghi *et al*. (2012) and 81.5 % was detected by Khatab and Samah (2013).

According to ISSR analysis the relationships among all genotypes were measured by a UPGMA cluster analysis of genetic similarity matrices, depending on the Nei-Li's similarity coefficient matrices cluster analysis was achieved and revealed that, L1 and L5 were the most similar genotypes and having of 0.74 similarity value, while Giza 123 and L4 had the lowest value (0.45) (Table 5).

Based on dendrogram of genetic distant among all genotypes it distributed them into two main clusters, only the lowest salinity tolerant (L4) genotype was found in the first one. While the second cluster divided into two sub-clusters, the first sub-cluster included sensitive check variety (Beecher), the second sub-cluster subdivided into two main groups, the first one included L1, L5 and L3 had MST, the other group contained the two highest tolerant genotypes (Giza 123 and L2) genotypes (Fig. 2). The dendrogram in the current study showed very net model of clustering according to salinity tolerance which revealed that ISSR primers were able to distinguish tolerant genotype. Similar results were recorded in barley by Khatab and Samah (2013)

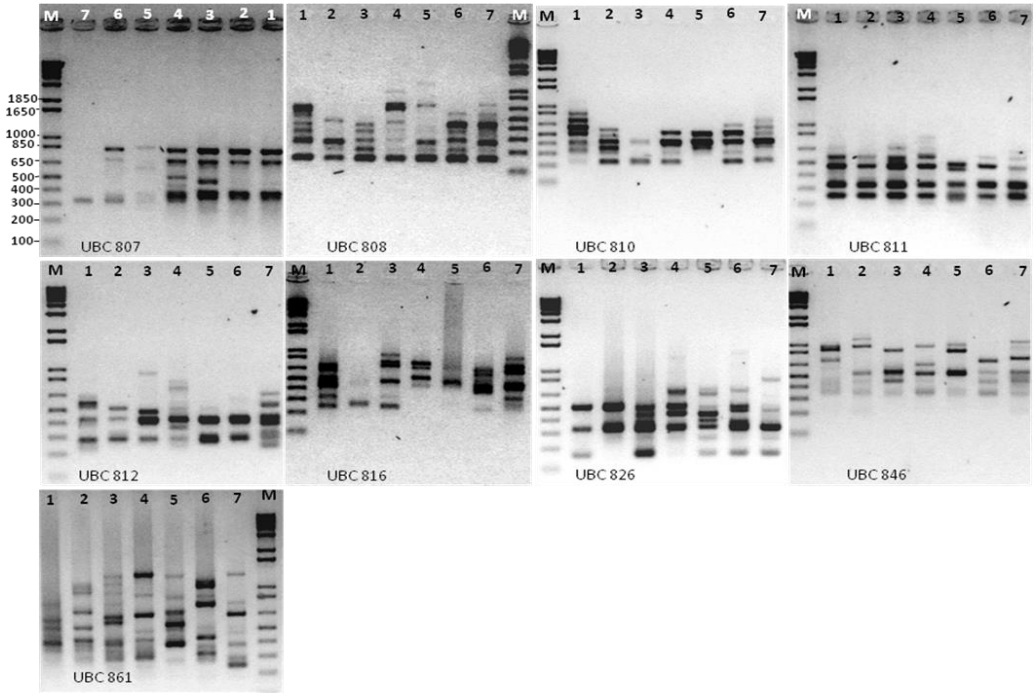

Figure 1. ISSR-PCR amplified fragments (amplicons) produced by nine primers for five barley landraces and two check varieties (1, Giza 123; 2, L1; 3, L2; 4, L3; 5, L4; 6, L5; 7, Beecher). M, DNA marker.

Table 4. Polymorphism obtained by nine ISSR primers in five barley landraces and two check varieties genotypes.

Primers	Primer sequence	Range of fragment size bp	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	Polymorphism %
UBC 826	$(AC)_8C$	155-844	9	1	8	88.88
UBC 812	$(GA)_8A$	266-939	11	2	9	81.81
UBC 846	$(CA)_8RT$	455-1178	8	0	8	100.00
UBC 807	$(AG)_8T$	340-833	7	1	6	85.71
UBC 808	$(AG)_8C$	160-700	7	2	5	71.43
UBC 810	$(GA)_8T$	131-632	8	1	7	87.50
UBC 811	$(GA)_8C$	250-550	6	2	4	66.67
UBC 816	$(CA)_8T$	228-880	9	0	9	100.00
UBC 861	$(ACC)_6$	226-1240	13	0	13	100.00
Total		131-1240	78	9	69	
Average			8.7	1	7.7	86.89

Table 5. The similarity index among five barley landraces and two check varieties based on ISSR

Genotypes	Giza 123	L1	L2	L3	L4	L5
L1	0.62					
L2	0.68	0.65				
L3	0.63	0.71	0.66			
L4	0.45	0.52	0.51	0.60		
L5	0.67	0.74	0.70	0.63	0.56	
Beecher	0.62	0.56	0.57	0.55	0.50	0.62

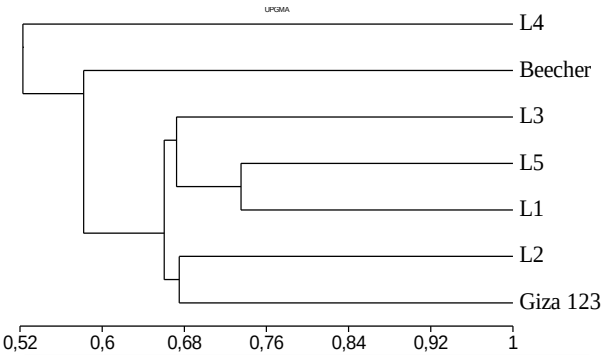

Figure 2. The dendrogram of genetic distances among all five barley landraces and two check varieties using UPGMA cluster analysis of Nei-Li's similarity coefficient based on ISSR markers.

CONCLUSION

In this study, five barley landraces genotypes and two check varieties were evaluated for their ability to gradually salinity stress tolerance and genetic diversity via evaluation of seedlings ability to death resistance under stress condition and ISSR molecular marker analysis, respectively. Significant differences were observed among them in their ability to survive under gradually salt stress condition. Whereas, L2 genotype had the highest seedling survival rate and was superior over salt tolerant check variety (Giza 123). Moreover, by using SSI analysis all genotypes were categorized into three groups, high, moderate and low salinity stress tolerant group. Furthermore, ISSR molecular analysis had an efficient ability to detect genetic variability among all varieties. In addition, they were properly able to distinguish the highest salinity tolerant varieties. However, our results suggested that landraces could be a beneficial source for genetic variability to obtain barley tolerant lines in breeding program.

REFERENCES

AERMAE, 2011. Agriculture Economic Report, Ministry of Agriculture, Egypt.

Akladious S. A. and Abbas S. M. (2014). Inter simple sequence repeat (ISSR) markers and some physiological attributes of barley (*Hordeum Vulgare* L.) genotypes to drought and potassium nutrition. J. Anim. Plant Sci., 24:620-633.

Ben El Maati F., Jlibéne M. and Moumni M. (2004). Study of the polymorphism of common wheat using ISSR markers. J. Food Agric. Environ., 2: 121-125.

Ceccarelli S. (1994). Specific adaptation and breeding for marginal conditions. Euphytica, 77: 205-219.

Dadashi M. R. (2008). Salinity Effects on Seedling Growth and Yield Components of Barley. Res. J. BioI. Sci., 3: 812-820.

Der G. and Everittt B. S. (2009)."Basic Statistics using SAS Enterprise Guide". J. Royal Statistical Society: Series A (Statistics in Society) 172 (2).

El-Awady M. A. H., El-Tarras A. A. E. and El-Assal S. E. (2012). Genetic Diversity of Some Saudi Barley (*Hordeum Vulgare* L.) Landraces Based on Two Types of Molecular Markers. Am. J. Applied Sci., 9: 752-758.

El-Hamamsy S. M. A. and Behairy R. T. (2015). Effect of Salinity Stress on Seedling Vigor and Biochemical Characters of Egyptian Barley Landraces (*Hordeum vulgare* L.). Middle East J. Appl. Sci., 4: 786-796.

Eshghi R., Abrahimpour F., Ojaghi J., Salayeva S., Baraty M. and Rahimi M. (2012). Evaluation of genetic variability in naked barley (*Hordeum vulgare* L.). Intl. J. Agri. Crop. Sci., 4: 1166-1179.

Fernández M.E., Figueiras A. M. and Benito C. (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor. Appl. Genet., 104: 845-851.

Fischer R. A. and Maurer R. (1978). Drought resistance in spring wheat cultivars. 1. Grain yields responses. Australian J. Agric. Res., 29: 897-912.

Foyer C. H. and Noctor G. (2005). Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses. Plant Cell, 17: 1866-1875.

Hori K., Sato K., Nankaku N. and Takeda K. (2005). QTL analysis in recombinant chromosome substitution lines and doubled haploid lines derived from a cross between *Hordeum vulgare* ssp. *vulgare* and *Hordeum vulgare* ssp. *Spontaneum*. Mol. Breed., 16: 295-311.

Hou Y., Yan Z., Wei Y. and Zheng Y. (2005). Genetic diversity in barley from west China based on RAPD and ISSR analysis. Barley Genetics Newsletter, 35:9-22.

Kendirli B., Cakmak B., and Ucar Y. (2005). Salinity in the Southeastern Anatolia Project (GAP), Turkey: Issues and Options. Irrigation and Drainage, 54: 115-122.

Khatab I. A. and Samah M. A. (2013). Development of Agronomical and Molecular Genetic Markers Associated with Salt Stress Tolerance in Some Barley Genotypes. Curr. Res. J. Biol. Sci., 5: 198-204.

Khodarahmpouret Z., Choukan R., Bihamta M. R. and Hervan E. M. (2011). Determination of the best heat stress tolerance indices in maize (*Zea mays L.*) inbred lines and hybrids under khuzestan province conditions. J. Agr. Sci. Tech., 13: 111-121.

Nei M. and Li W. H. (1979). Mathematical model studying genetic variations in terms of restriction endonucleases. Proc. Nat. Acad. Sci., 76: 5269-5273.

Newman C. W. and Newman R. K. (2006). A brief history of barley foods. J. Cereal Foods World, 51: 4-7.

Newton A. C., Aker T., Baresel J. P., Bebeli P., Bettencourt E., Bladenopoulos K. V., Czembor J. H., Fasoula D. A., Katsiotis A., Koutis K., Koutsika-Sotiriou M., Kovacs G., Larsson H., Pinheiro de Carvalho M. A. A., Rubiales D., Russell J., dos Santos T. M. M. and Vaz Patto M. C. (2010). Cereal landraces for sustainable agriculture: a review. Agron. Sustain. Dev., 30: 237-269.

Parida A. K. and Das A. B. (2005). Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety, 60: 324-349.

Sbei H., Shehzad T., Harrabi M. and Okuno K. (2014). Salinity Tolerance Evaluation of Asian Barley Accessions (*Hordeum vulgare* L.) at the Early Vegetative Stage. J. Arid Land Studies, 24: 183-186.

Tabatabaei S. A. (2013). Changes in proline, protein, catalase and germination characteristics of barley seeds under salinity stress. Intl. Res. J. Appl. Basic. Sci., 5: 1266-1271.

Tiwari J. K., Munshi A. D., Kumar R., Pandey R. N., Arora A., Bhat J. S., and ureja A. K. (2010). Effect of salt stress on cucumber: Na+/K+ ratio, osmolyte concentration, phenols and chlorophyll content. Acta Physiol. Plant, 32: 103-114.

Vaidyanathan H., Sivakumar P., Chakrabarsty R. and Thomas G. (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.)-differential response in salt-tolerant and sensitive varieties. Plant Sci., 165: 1411-1418.

Yousofinia M., Ghassemian A., Sofalian O. and Khomar S. (2012). Effects of salinity stress on barley (*hordeum vulgare*, l.) Germination and seedling growth. Intl. J. Agri. Crop. Sci., 4: 1353-1357.

تقييم بادرات بعض سلالات الشعير البرية تحت ظروف الملوحة والتحليل الجزيئي

طلعت بشندي

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الملوحة هي واحدة من أكبر التحديات التي تحد من إنتاجية المحاصيل. لذلك العمل على إنتاج أصناف أكثر تحملا للملوحة للملوحة يمثل أولوية قصوى للمربين. تعتبر السلالات البرية من أهم مصادر التباين الوراثي التي يمكن استخدامها في تحسين أصناف جديدة. لذلك في هذه الدراسة تم الحصول علي خمسة سلالات برية من مواقع مختلفة في محافظة الوادي الجديد بمصر بجانب صنفين أحدهما متحمل (Giza 123) والأخر حساس للملوحة (Beecher) للمقارنة ككنترول. حيث تم تقييمها لمدي قدرتها علي تحملها للإجهاد الملحي والتباين الوراثي بينهما وذلك عبر تعريض البادرات لسلسلة تركيزات متدرجة متوالية من الملح وأيضا باستخدام تحليل الواسمات الجزيئية (ISSR). حيث تم إحصاء البادرات التي كانت على قيد الحياة تحت كلا من الظروف العادية والإجهاد الملحي. وأظهرت النتائج وجود فروق معنوية بين التراكيب الوراثية تحت ظروف الإجهاد الملحي. حيث تأثرت جميع التراكيب الوراثية بالملوحة و تباين موت البادرات بينها، حيث كان الصنف Beecher أكثرها تضررا، وكانت السلالة L2 أقلها موتا للبادرات متفوقة بذلك علي الصنف جيزة 123. وعلاوة على ذلك استخدمت الواسمات الجزيئية (ISSR) لدراسة التنوع الوراثي ومدي القرابة بين هذه السلالات. حيث تم الحصول علي نسبة 86.89 ٪ لتعدد الأشكال المظهرية. و كانت السلالتان L1 وL5 أكثرها تشابها (0.74) بينما كان أدنى تشابه (0.45) بين السلاله L4 و الصنف جيزة 123. وعلاوة على ذلك استطاع التحليل العنقودي وقياس القرابة الوراثية الي فصل التراكيب الوراثية الأكثر تحملا للملوحة في مجموعة واحدة. تفوق السلالة L2 مقارنة بالكنترول يشير إلى أن السلالات البرية يمكن أن تكون أداة مفيدة للتحسين الوراثي لإنتاج سلالات شعير أكثر تحملا للملوحة.